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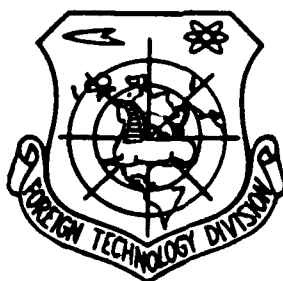
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H⁺-ISFET TYPE PENICILLINASE SENSOR

by

Zhong Lichan, Li Gaoxiang, et al.



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H^+ -ISFET TYPE PENICILLINASE SENSOR

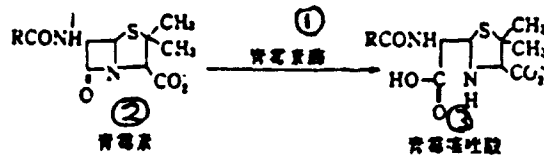
Zhong Lichan Li Gaoxiang Wang Zhengxiao Liu Luna

We took an SOS type H^+ -ISFET and pentyl dialdehyde connective bovine blood serum protein- penicillinase membrane set and synthesized a single transistor output type penicillinase- H^+ -ISFET sensor probe (below simply designated penicillinase FET). In conjunction with this, it was used to measure the amount of penicillin contained in solutions. The penicillinase FET's degrees of reactive sensitivity in 0.005mol/L, 0.01mol/L, and 0.02mol/L phosphate buffer were, respectively, 11-12mV/m mol/L, 7.5-8.0mV/m mol/L, and 3.7-4.0V/m mol/L. The reaction time was 30s. In 0.02mol/L phosphate buffer, the standard curve linearity range was 0.5-25mmol/L. The correlation coefficient is 0.9976. This penicillinase, after 8 repetitions of measurments in 0.01mol/L phosphate buffer with a penicillin concentration of 10mmol/L had a standard deviation (SD) of 1.67mV. The coefficient of variation was 2.1%. The storage life was greater than 4 months. Its life in use was over 1 month (making one test each day).

KEY TERMS Ion Sensitive Field Effect Transistor; Penicillinase; Immobilized Enzyme; Penicillin ENFET Sensor

Ion sensitive field effect transistors (below simply referred to as ISFET), as well as the biologically sensitive field effect transistors which have been developed on this basis, are a new type of microsemiconductor chemistry instrument which has been developed in recent years. They compare ion selection electrodes and enzyme electrodes to each other. They possess small volumes. The reactions are fast. Their ouput resistance is low and other similar special characteristics. They have drawn attention from relevent fields inside China and outside. The speed with which their test manufacture was begun is just now in the process of being speeded up^[1].

Among ISFET's, hydrogen ion sensitive field effect transtistors (that is, H^+ -ISFET) are a very important type. A great number of biological materials, for example, glucose, penicillin, urea, and so on, in their corresponding enzymes, are involved in catalysis of reactions which are always accompanied by changes in the pH values of solutions. A case in point would be:



(1) Penicillinase (2) Penicillin (3) Penicillin (illegible) Thiozolate

As far as the changes in pH values of solutions which are caused in this reaction are concerned, these changes and the amounts of penicillin contained in the solutions are in direct proportion to each other. As a result of this, it is possible to directly measure the amounts of penicillin contained in the solutions.

During the process of fermenting penicillin, it is necessary to go through check out measurements of the concentration of the penicillin. At the present time, normally, one opts for the use of penicillin concentration check measurement methods. Examples would be iodine measurement methods, but the procedures are tedious and overelaborate, they consume a great deal of time, and their precision is not very high, either. Because of this, it is necessary to have a fast convenient method in order to replace the iodine measurement methods. From the 1970's onward, with regard to work in the area of penicillinase electrodes, there have been a number of reports both inside China and outside [2-7]. As far as research in the area of penicillinase FET's is concerned, one has only seen reports outside of China [8-9].

Going through research, we manufactured a penicillinase FET sample transistor which had relatively good capabilities. In conjunction with this, it was possible to use it in order to quickly and easily make precise measurements of the amounts of penicillin contained in solutions. For these devices, the reaction sensitivities, reaction times, linearity ranges of standardization or calibration curves, and other similar aspects of their capabilities are basically in line with those that were reported in references outside of China. The storage stability aspects were better than

those of the same type of devices reported in references outside of China.

MATERIALS AND METHODS

(I) Materials

Bovine serum protein or albumin (BSA) is a product of the Chinese Academy of Sciences' Biochemistry Research Institute. Pentyl dialdehyde (25%) was imported from E. Merck and divided up for separate use. Penicillinase (waxy sporulation bacillus -endoacylaminase, 40IU/mg) was provided by the Institute of Medical Sciences' Medical Pharmacology Biotechnology Research Institute. The rest of the reagents used were all analytically pure. The phosphate buffer solution had a pH of 7.2.

(II) Device Structure

The penicillin-ENFET probe's structure is basically, on top of a grid sensitive area of H^+ -ISFET, a covering layer of immobilized penicillinase membrane. The actual structure is as shown in Fig.1. From Fig.1, it is possible to see that, for the probe's lead wires, a choice was made for the use of printed circuits. For connections, a choice was made for the use of plugs. Fig.2 is a display diagram of a longitudinal cross section of the probe's chip section.

(III) Device Manufacture

1. SOS type H^+ -ISFET manufacture and its special characteristics. H^+ -ISFET's opt for the use of a relatively advanced SOS (Silicon-on-Sapphire), that is, blue sapphire with silicon extending out beyond it used in technical manufacture. The special characteristics of its typical form are as follows. Degree of response sensitivity is 50mV/pH. Its linear correlation coefficient (pH=3-9) $r \geq 0.999$. Its response time < 1 s. For valence I positive ions (Na^+ , K^+) the selection coefficient $< 10^{-4}$. Under stable operational conditions, drift < 1 mV/h.

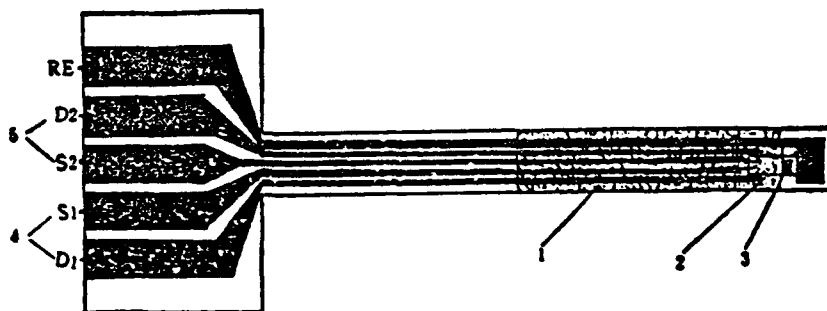


Fig.1 Structure diagram of penicillin-ENFET probe

1. 密封材料 Sealing material 2. 管芯 Chip 3. 敏感区 Sensitive area 4. 管1 FET 5. 管2 FET

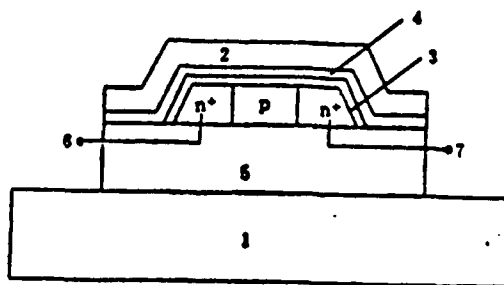


Fig.2 Cross section diagram of the chip of penicillin-ENFET probe

1. 基板 Supporting plate 2. 青霉素酶膜 Penicillinase membrane 3. SiO₂层 SiO₂ layer 4. Si₃N₄层 Si₃N₄ layer 5. 蓝宝石衬底 Sapphire substrate 6. 源极 Source 7. 漏极 Drain

2. Immobilized Enzyme Membrane Formation. Immobilized penicillinase membranes on ISFET grid electrodes are such that they opt for the use of pentyl dialdehyde-bovine serum albumin coupling for their manufacture. In order to cause the enzyme membrane and the grid electrode surface to be firmly bonded together, one first takes FET and introduces them into 10% pH 7.0 type 'γ'-aminopropyltriethoxysilane aqueous solution. At 50°C, they react for 2-3 h. Following that, one uses water to rinse them clean and air dries them. Using 0.02mol/L pH 7.2 type phosphate buffer solution, one takes bovine

serum albumin and penicillinase and blends them together into a solution as well as pentyl dialdehyde, blending it into a 2.5% aqueous solution. One takes these three types of solutions in equal volumes and mixes them uniformly, after which, one uses micro amounts to carry out sampling, taking one drop of the mixture and putting it onto the grid electrode surface of the FET. At room temperature, 15 min after coupling, it is immersed into a 0.1mol/L lysine solution for 10 minutes. After that, the reaction stops. Finally, one uses 0.01mol/L pH 7.2 type phosphate buffer solution to rinse it clean and, after that, in the same type of buffer solution, one stores it at 4°C.

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(IV) Test Equipment, Circuitry, and Methods

The special nature of penicillin-ENFET is such that, in a 10ml beaker, using an electromagnetic stirring device at a constant speed of stirring, one takes calomel or mercurous chloride electrodes to be the reference electrode to carry out measurements. Into the beaker, one adds 4ml phosphate salt buffer solution and 1ml of a different concentration of penicillin (using the same type of buffer solution in the preparation), recording within 1 minute the change in output voltage.

The special type of measurement circuitry in these devices is as shown in Fig.3. In the Fig., Ref is the reference electrode (for example, calomel or mercurous chloride electrodes, silver chloride silver electrodes, or other similar types). I_0 is the constant current source. The ISFET or biologically sensitive FET's leakage source current $I_{DS}=I_0$. The leakage source voltage $V_{DS}=I_0 \cdot R$. The devices operate in a state of constant current and constant voltage. If the concentration of penicillin shows a change, the solution-penicillinase membrane and grid surface Si_3N_4 membrane boundary surface electrical potentials are different, and there is then a change. In this way, there will then be an adjustment in the penicillin-ENFET's channel conductance, causing the device's leakage source current, I_{DS} , to show an increasing or decreasing trend. However, due to the fact that I_{DS} is a constant value, in this way, one then makes things so that the source electrical potential will correspondingly increase or decrease, in order to maintain the constant value of I_{DS} . Due to the fact of the source electrode going through following devices and output terminal connections, as a

result, in the solutions, changes in the concentration of penicillin will then be expressed as changes in the output electrical voltage V_{out} .

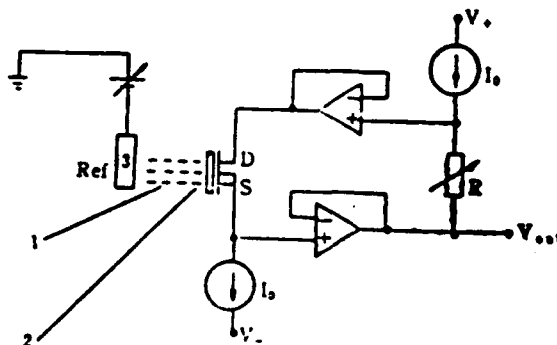


Fig.3 Circuit diagram for determining the characteristics of penicillin-ENFET
1. 溶液 Solution 2. 酶膜 Enzyme membrane
3. 参比电极 Reference electrode

RESULTS AND DISCUSSION

(I) Penicillin-ENFET Time Response Curves

Fig.4 shows the curves for the changes over time in the output voltages ΔV (mV) of penicillin-ENFET in solutions with different concentrations of penicillin. From the Fig., it is possible to see that, generally, around 30s, the curves have already tended toward gentle slopes.

(II) The Influence of Buffer Concentrations on Penicillin-ENFET Degrees of Reactive Sensitivity

Fig.5 is the relationships between the output voltages ΔV (mV) of penicillin-ENFET for different concentrations of phosphate buffer solution and the concentration of penicillin. From Fig.5, it is possible to obtain relationships such as those set out in Table 1.

Table 1 Sensitivity and linear range of calibration^a curve of penicillin-ENFET in different concentrations of phosphate buffer at pH7.0

Concentration of phosphate buffer(mol/L)	0.005	0.01	0.02
Response sensitivity (mV/mmol/L)	11—12	7.5—8.0	3.7—4.0
Linear range (mmol/L)	0.5—10	0.5—13	0.5—25
Linear correlation coefficient (r)	0.9983	0.9979	0.9976

The lower limit of the penicillin-ENFET calibration curve linearity range is 0.5mmol/L. Its upper limit goes up with increases in the concentration of buffer solution used at the time of test measurements. However, the degree of sensitivity drops. The reason for this is that buffer solutions with high concentrations have relatively strong buffering capabilities. As a result of this, they cause enzyme reactions to be carried out at constant pH. Because of this, the upper limit of the linearity range goes up. However, at the same time, relatively strong buffering capabilities also lead to a drop in the degree of reaction sensitivity of the sensors in question. To put it the other way around, in situations where buffer solution concentrations are low, the degrees of reactive sensitivity of sensors are high. However, following a drop in the concentration of buffer solutions, there is the causing of a drop in enzyme activity as well as the causing of a drop in the upper limit of the linearity range.

(III) Penicillin-ENFET Reproducibility in Penicillinase Reactions

Fig.9(a) is the results for penicillin-ENFET from the carrying out of eight repetitions of measurements in 10mmol/L penicillin solutions made up with 0.01mmol/L phosphate buffer solutions. The

output voltages for the eight repeated measurements were all within $78 \pm 2-3\text{mV}$. Their standard deviation (SD) was 1.67 mV . The coefficient of variability (CV) was 2.1% . Besides this, we also measured, in 0.01mmol/L phosphate buffer solution, penicillin concentrations from low to high ($1 \rightarrow 20\text{mmol/L}$). And, again, the changes from high to low ($20 \rightarrow 1\text{mmol/L}$) when there were changes in output voltages from the sensor devices in question. As is shown in Fig.6(b), there was almost complete congruence of voltage output values obtained when there were changes in upper and lower lines of penicillin concentrations.

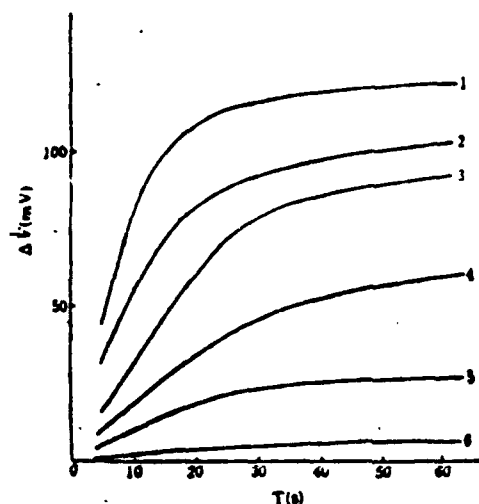


Fig.4 Time response curves of penicillin-ENFET to different concentrations of penicillin solution

青霉素浓度 Penicillin concentration (mmol/L),
1. 15.0, 2. 10.0, 3. 8.0, 4. 5.0, 5. 2.0, 6. 0.2;
磷酸缓冲液浓度 Phosphate buffer conc.(mol/L),
0.005; 测量温度 Temperature(°C): 20

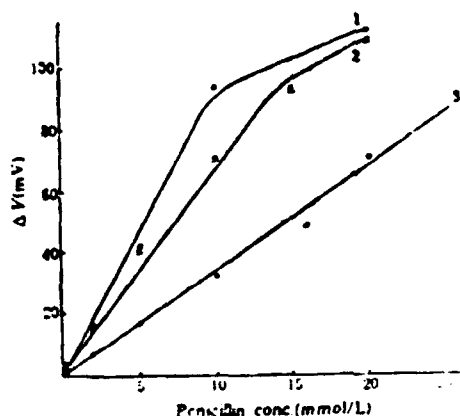


Fig.5 Calibration curves of penicillin-ENFET in different concentrations of phosphate buffer
缓冲液浓度 Buffer conc.(mol/L): 1. 0.005, 2. 0.01,
3. 0.02

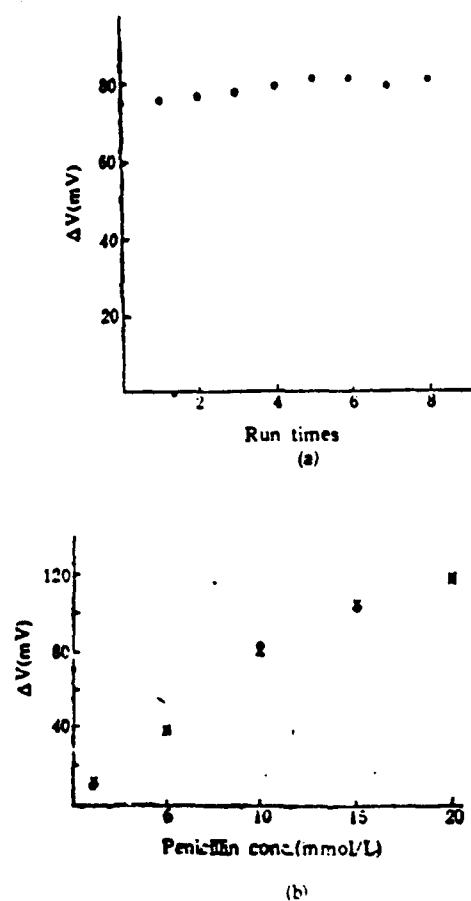


Fig.6 Reproducibility of response of penicillin-ENFET to penicillin

(a) 在同一浓度青霉素溶液中测定

Performed in phosphate buffer with same concentration penicillin

(b) 在不同浓度的青霉素溶液中测定

Performed in phosphate buffer with different concentrations of penicillin

○青霉素浓度由低向高 With penicillin concentration increasing

×青霉素浓度由高向低 With penicillin concentration decreasing

(IV) Penicillin-ENFET Probe Storage Stability

One takes the penicillinase-FET probe and stores it at 4°C in a 0.01mol/L phosphate buffer solution. Within a certain period, one measures the effect on a 10mmol/L penicillin solution (using 0.01mol/L phosphate buffer solution to make it up). The results of the

measurements clearly show that the probe in question, under the conditions discussed above, after being stored for 4 months, had output voltages which had only slightly dropped. Table 2 is the storage stability data for penicillin-ENFET.

表 2 青霉素-酶FET的贮存稳定性
Table 2 Storage stability of penicillin-ENFET

贮存天数 Store days	1	14	21	48	57	61	69	71	79	82	86	97	105	122
输出电压 Vout(mV)	75.7	71	70	70	71	71	69	69	69	71	70	61	68	74

此数据是在室温下测得的, 温度范围为22—29℃
The data were determined at room temperature
ranged from 22—29℃

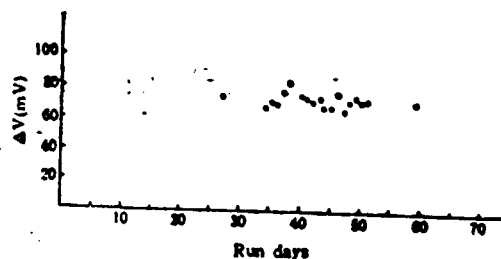


图 7 青霉素-酶FET的操作稳定性
Fig.7 Operation stability of penicillin-ENFET
在室温下测定, 温度变化范围为22—29℃
Determined at room temperature ranged from
22—29℃

(V) Penicillin-ENFET Useful Life

In a 0.01mol/L phosphate buffer solution, measurements were taken of the effects of penicillin-ENFET on 10mmol/L penicillin solutions. Each day they were utilized once. Between each iteration of measurements, one took sensors and stored them at 4°C in 0.01mol/L phosphate buffer solution. Fig.7 is the operating stability of penicillin-ENFET. After the sensors in question were manufactured, on the basis of the storage conditions described above, after being stored for 28 days, test measurements were begun each day. In the Fig., the wave undulations of the output voltages may possibly be due to wave undulations of the room temperatures at the times of the measurements. From Fig.7, it can be seen that, under the operating conditions described above, penicillin-ENFET are capable of operating stably for more than 30 days.

At the present time, the penicillin-ENFET which have been test manufactured are only single transistor output type sensor devices. When measurements are taken of amounts of penicillin contained in solutions, it is still necessary to make use of calomel or mercurous chloride electrodes to act as reference electrodes. Because of this, one still finds in existence the shortcomings of large volume and the interference of such environmental factors as temperature, light, and

other similar factors on measurements. In order to overcome these shortcomings, we will continue the test manufacture of double transistor separate output type penicillin-ENFET which carry reference electrodes in order to achieve in them small volume and even better capabilities than those of penicillin-ENFET.

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